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(54) METHODS FOR TAGGING AND TRACING MATERIALS WITH NUCLEIC ACIDS

VERFAHREN ZUR MARKIERUNG UND ZUM NACHWEIS VON STOFFEN MIT NUKLEINSÄUREN

**PROCEDES D'ETIQUETTAGE ET D'INDICATION DE MATIERES A L'AIDE D'ACIDES
NUCLEIQUES**

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(72) Inventor: **DOLLINGER, Gavin, D.**
San Francisco, CA 94114 (US)

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EP-A- 0 201 184	EP-A- 0 231 608
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(73) Proprietor: **F. HOFFMANN-LA ROCHE AG**
4002 Basel (CH)

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Description

The present invention provides methods for tagging and tracing materials using nucleic acids as taggants. The process of tagging involves altering a substance in a manner that allows for the subsequent identification of the substance by detecting the alteration. The alteration disclosed herein involves nucleic acids.

Society currently attempts to track the manufacture and distribution of a large number of diverse substances, including (1) natural resources such as animals, plants, oil, minerals, and water; (2) chemicals such as drugs, solvents, petroleum products, and explosives; (3) commercial by-products including pollutants such as radioactive or other hazardous waste; and (4) articles of manufacture such as guns, type writers, automobiles and automobile parts. Tagging aids in the determination of product identity and so provides information useful to manufacturers and consumers.

Some of the diverse uses of tagging methods and reagents include the identification of the manufacturer of an explosive, even after detonation, and the determination of flow patterns, so as to measure the spread of pollutants. The present invention provides a significant advance in the field, because the taggants can encode substantial amounts of information to aid subsequent identification. Moreover, by using recently available amplification technology, one can detect far less taggant than ever before. In fact, the present tagging methods work with such vanishingly small levels of taggant that drugs tagged by the present method can still pass the FDA standards (10 pg/dose) for amount of DNA, in this case, taggant DNA, in any product.

The use of tagging substances with polypeptides is known. U.S. Patent Nos. 4,359,353 and 4,441,943. As with the nucleic acid taggants of the subject invention, the polypeptide taggants use the order of amino acids in the polypeptide to encode information.

European Patent Publication No. 231 608 describes a process for labeling a micro-organism by integrating in its genome a specific DNA sequence of at least 15 nucleotides. PCT Patent Publication No. 87/06383 describes a process of labeling articles it is wished to authenticate by means of a signal sequence of a nucleic acid having from 1000 to 10,000 nucleotides.

The present invention provides a method for tagging a material (any substance) by treating the material with a nucleic acid taggant so that said nucleic acid attaches to said material in an amount sufficient for subsequent detection. The nucleic acid taggant comprises a specific nucleotide sequence or has a distinct composition of specific nucleotides to facilitate tracing. The present invention also provides a method for tracing a material tagged with a nucleic acid taggant that comprises determining the presence of a nucleic acid sequence specific for said taggant in the material. In a preferred embodiment, the tracing method also comprises treating the material under conditions that would result in the amplification of a nucleic acid sequence of a taggant, if present, to determine if the specific nucleic acid sequence is present.

More particularly, this invention provides for a method of monitoring the presence of a substance released into the environment or the stream of commerce, as defined in the appending claims. An additional step of amplification of the nucleic acid is also described herein. This amplification is typically done prior to detection and is preferably achieved using polymerase chain reaction technology. The nucleic acids can be either naturally occurring or synthetically derived. Preferred synthetic nucleic acids are comprised of inosine bases or 7-deazo-2'-deoxyguanosine nucleotides.

The materials or substances of this invention include those selected from the group consisting of air pollutants, oils, aromatic compounds, explosive compositions, food stuffs, medicaments, inks, paper goods, and paint products. The nucleic acids can be optionally bound to a component of the tagged substance through a covalent bond. "Covalently bound to a component of the substance," means that the nucleic acids are covalently bound to the tagged material or component thereof. Where the material is comprised of different components such as different chemical compounds, the nucleic acid may be covalently bound to any one or all of the components.

The nucleic acids used as taggants may be free or they may be covalently bound to a solid support (such as latex beads, dextran or magnetic beads) which is then mixed with the material being tagged. Alternatively the nucleic acids may be encapsulated by polymeric substances (such as proteins) or by lipophilic compositions (such as liposomes).

This invention also provides a method for tagging substances as defined in the appending claims. The preferred taggants and substances are as described above.

The following definitions are provided to aid understanding of the invention:

SUBSTANCE - a material that can be tagged and traced according to the method of the invention.

NUCLEIC ACID - a deoxyribonucleotide or ribonucleotide polymer in either single- or double-stranded form; modified nucleotides may comprise all or part of the nucleic acid.

TAGGANT - a nucleic acid that comprises a specific nucleotide sequence or has a specific nucleotide composition, the specificity of said sequence or composition providing a means to store information. Taggants are typically non-biologically functioning in that they are not a part of a functional nucleic acid sequence operating in a living cell. Those of skill will recognize that living organisms contain unique nucleic acid sequences either natural or artificially introduced. This invention is not meant to embrace these naturally occurring and biologically functional forms of taggants. Taggants are generally added to substances. They are not a functioning part of the substance.

TAGGING - the process of treating a material with a composition, the taggant, for subsequent identification of the material by detection of the taggant.

TRACING - the process of determining the origin or source of a material.

This invention relates to the use of nucleic acids for monitoring, detecting and tracing substances released into the environment or released into the stream of commerce. The nucleic acids are used as an additive to tag such substances. The specifics of compounding the nucleic acid as a taggant and its subsequent recovery for analysis is dependent upon the specific substance into which the nucleic acid is being added. The following guidelines are offered.

Nucleic acids.

The nucleic acids for use in this invention can provide a limitless amount of information. By using combinations of universal sequences (accepted as industrial standards) and by varying levels of specific sequences, one can identify the type of generic product, the product's origin (company specific sequences), the lot or batch, and even provide an identifier for a unit of commerce.

The nucleic acids suitable for use in this invention include both natural and non-natural nucleic acids. They can be single or double stranded. Natural nucleic acids refer to polymers of either DNA or RNA including the 5 naturally occurring bases, adenine, thymine, guanine, cytosine and uracil.

Non-natural or synthetic nucleic acids can also be used in this invention. Synthetic nucleic acids in some instances have advantages over natural nucleic acids, e.g. in stability, solubility, etc. Some synthetic nucleotides are less likely to be degraded by nuclease activity, by chemically active substances or by environmental conditions such as heat or ultraviolet radiation. The use of non-natural nucleotides is limited only by their ability to be effectively detected by the selected detection means. For tagging methods using the preferred PCR technology, the synthetic nucleic acid must form duplexes with the primers and function as a template for the polymerases used in the procedure. Non-natural nucleic acids for use in this invention include those incorporating inosine bases, and derivatized nucleotides, such as 7-deazo-2'-deoxyguanosine, methyl- (or longer alkyl-) phosphonate oligodeoxynucleotides, phosphorothioate oligodeoxynucleotides, and alpha-anomeric oligodeoxynucleotides.

The nucleic acids for use in this invention can provide a limitless amount of information. By using combinations of universal sequences (accepted as industrial standards) and by varying levels of specific sequences, one can identify or authenticate the type of generic product, the product's origin (company specific sequences) and the lot or batch.

Tagged Substances.

The following substances are not meant to be limiting, but will serve to offer those of skill an understanding of the versatility of this invention. For example, it is expected that this invention will find application in explosives (such as plastic explosives and gunpowder), aerosols (such as automobile or industrial pollutants from smoke stacks), organic solvents (such as from dry cleaners, chemical factories, airports, and gas stations), paper goods (such as newsprint, money, and legal documents), inks, perfumes, and pharmaceutical products (such as medicaments).

Preparation of the nucleic acids will vary in accordance with the nature of the substance and upon the expected impact of the environment in which the substance is expected to be placed. The substances can be classified as solids, liquids or gases. The substances can be either inert or active, with liquids and aerosols being further divided as being either polar or non-polar.

Inert solids such as paper, many pharmaceutical products, wood, some foodstuffs, or polymer compounds (e.g., plastics), can be processed with the taggant or the taggant can be sprayed onto the surface of the solid. Taggants can be physically mixed with inert liquids or gases.

Chemically active substances, such as foodstuffs with enzymatic activity, polymers with charged groups, or acidic pharmaceuticals may require that a protective composition be added to the nucleic acid taggants. Protective compositions would include substances which would encapsulate the nucleic acids and protect them from enzymatic or chemical degradation. Liposomes are a technology presently available for use as a protective capsule for nucleic acids. Typical liposomes are micelle bodies formed by detergents. Alternatively polymeric substances can be used to electrostatically bind to and encapsulate the nucleic acids. Polymeric substances would include proteins such as virus coat proteins.

The same approach is used for the addition of taggants to chemically active liquids except the protective composition for a liquid is preferably compatible with the liquid's polarity. The compatibility of taggant with a liquid substance is preferred for both inert and chemically active liquids. For example, oils and other non-polar liquids can be tagged effectively by the use of detergents added to the taggant prior to the addition of the taggant to the liquid. One could rely upon Brownian motion to ensure a uniform distribution in the liquid.

For gases, the taggants are simply mixed with the gas. This is because the taggant is much smaller than what is usually considered to be dust (0.2 μ m) particles. Containerized gases would have the taggants placed in the container.

For gases being released into the atmosphere, the taggants could be mixed before release or at the time of release. For example, to track the pattern of dispersal of gases released by industry, one could attach an aerosol delivery device to an exhaust outlet and introduce a metered amount of taggant as the gas is released.

The use of nucleic acid taggants for the tracing of radioactive products, including waste, is anticipated. The means for tracing would be as described herein for non-radioactive materials with the use of appropriate protection. It should be noted that short oligonucleotides of less than 1000 bases are preferred due to their greater stability against degradation from radiation.

Recovery and detection of taggants.

The amount to add to a substance and preferred means for recovery and detection of nucleic acid taggant is dependent upon the substance being monitored. The means for detection and the amount of available sample being collected will dictate the amount of taggant required for each application of this invention. Therefore, in the absence of a means to amplify the taggant, the amount of taggant added would depend on the logistics of collecting enough substance to recover a detectable amount of taggant.

The preferred molecular structure of the nucleic acid taggant will vary with the means used to detect the nucleic acid. Typically at least 20 bases are necessary to ensure adequate specificity for any taggant so that accidental contamination will not lead to false results. Preferably, the sequence should comprise multiple regions of specificity. The longer the sequence, the more information may be carried. However, it will be recognized that the higher the information content of the taggant the more likely that degradation will become a problem. Typically, fragments under 1 kilobase are preferred.

Recovery of the taggant can be achieved by the use of standard techniques. Typically, the sample is washed or extracted with either distilled water or a buffered solution. Physiological pH is preferred as pH extremes may degrade nucleic acid. Charged substances might require washing in high molarity salt buffers to act as ion exchangers with the electrostatically bound nucleic acids. Detergents, either ionic or non-ionic, are helpful to remove nucleic acids from surfaces or from complex biological mixtures. Using phenol based extractions or phenol/chloroform extractions, one can recover nucleic acid from complex biological substances or from oil based substances.

The recovered nucleic acid can be concentrated by standard techniques such as precipitation with alcohol, evaporation, or microfiltration.

Because of the limits of sensitivity for the detection of nucleic acid, there is an obvious advantage to using methods for amplifying the recovered taggant. For example, using the polymerase chain reaction procedure [PCR] disclosed in U.S. Pat. Nos. 4,683,202 and 4,683,195 and European Patent Applications Nos. 258,017 and 237,362, one can amplify and detect nucleic acid molecule. The PCR method can be used with modification to amplify single stranded taggants, double stranded taggants and DNA or RNA taggants.

Those of skill in the art recognize that the PCR amplification can be carried out in a variety of ways. For instance, inverse PCR and asymmetric PCR are well known variations of the technique. In another variation, promoters for RNA transcription can be incorporated into primers, which, when extended and replicated by PCR, can then be used to create RNA copies of the target sequence. These RNA copies can, in turn, be several reverse transcribed into DNA, which can then be amplified by PCR. As with all PCR processes, reaction cycles can be repeated as often as desired.

For PCR, the preferred means for amplification, a double stranded taggant is preferred, although a single stranded taggant becomes a double stranded after the first cycle of amplification. The taggant is preferably a minimum of 60 bases long. This permits the hybridization of two primers which are preferably each 20 bases long, and which, when hybridized to the taggant, are separated by an internal region between the primer specific regions of the taggant of about an additional 20 bases.

When detecting nucleic acid by PCR, prior knowledge of the target sequence is necessary to provide appropriate primers. This necessary knowledge offers a valuable degree of security for those who desire it. For without the primers which can remain proprietary, the taggant can be virtually undetectable.

For detection of taggants, one can use standard nucleic acid hybridization assays or nucleic acid sequencing. The standard nucleic acid hybridization assays include single phase and mixed phase assays such as sandwich assays.

The specific detection procedure used is not critical to this invention. Nucleic acid hybridization technology is not a static art. New developments are anticipated and this invention is applicable to any new improvements. An overview of the state of the art can be found in Nucleic Acid Hybridization A Practical Approach, Eds. Hames, B.D. and Higgins, S.J., IRL Press, Wash. D.C., 1987.

The use of nucleic acid hybridization assays requires prior knowledge of the sequence being detected. Knowledge of the taggant's sequence permits the use of appropriate complementary oligonucleotides for capture or signal purposes.

Alternatively, the nucleic acids recovered from the samples can be sequenced using conventional sequencing technology. Commercially available kits are suitable for this purpose. The basic sequencing technology is derived from

seminal references such as the Maxam and Gilbert procedure for DNA sequencing described in Methods in Enzymology 65:(part 1) 497-559. Sequencing is a more difficult procedure to conduct, but offers greater reliability than nucleic acid hybridization assays. This is due to the possibility of contamination by extraneous nucleic acid with sufficient complementarity to hybridize to the selected probes and offer false positives.

Kits.

This invention also provides for a manufactured product such as a kit designed to tag and monitor substances. Such a kit would include nucleic acid taggants and polynucleotides which are complementary to the taggants. The complementary polynucleotides could be designed for use as signal probes, capture probes or as primers in the PCR method. The kits might also contain signal means such as enzymes, radio-isotopes, fluorescent labels and the like.

All cited references are provided for the convenience of the readers. The knowledge contained therein is well known to those of skill in the art.

The following examples are provided for illustrative purposes only and they are not to be interpreted as limitations of the invention.

Examples

The following examples all used a double stranded taggant of a sequence complementary to the DQ α allele 1.3. This sequence is derived from a rare DQ α type and has a reduced likelihood of being accidentally present as a contaminant in the materials described below. The taggant was also labelled with ³²P to follow experimentally the tag during the entire procedure. The unique sequence of one strand of this taggant has been provided in Table 1.

The taggant was recovered as described below. All amplifications were done with 40 cycles on a Perkins-Elmer Cetus Instruments thermal cycler using the protocols and reagents provided by the manufacturer. Biotinylated primers GH26 and GH27 having the sequence provided in Table 1 were used to initiate the PCR process.

Table 1. Nucleic acid sequences for DH α , related primers and probes.

GH26 - 5' GTGCTGCAGGTGTAAACTTGTACCAG 3'

GH27 - 5' CACGGATCCGGTAGCAGCGGTAGAGTTG 3'

Taggant sequence DQ α -

**5' TTTTACGGTCCCTCTGGCCAGTTCACCCATGAATTTGATGGAGATGAG
CAGTTCTACGTGGACCTGGAGAAGAAGGAGACTGCCTGGCGGTGGCCTGAGT
TCAGCAAATTTGGAGGTTTTGACCCGCAGGGTGCCTGAGAAACATGGCTGT
GGCAAAACACAACCTTGAACATCATGATTAAACGCTA 3'**

Capture probe GH89 5' - CTGGAGAAGAAGGAGAC - 3'

The Standard PCR procedure.

The following PCR procedure was used to amplify DQ α in each of the different substances illustrated below. The basic PCR mixture consists of:

10x TAQ buffer	Tris HCl pH 8.3 KCl MgCl ₂	10 μ l
100mM		
500mM		
15.0mM		
10 μ M GH26		1.5 μ l
10 μ M GH27		1.5 μ l
100mM dNTPs		0.75 μ l
5U/ μ l TAQ		0.5 μ l

(continued)

distilled water		35 μ l
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50 μ l of the basic PCR mixture is mixed with an equal amount of sample. Each cycle consists of 30 second incubations at 94°C followed by 30 sec at 55°C and 30 sec at 72°C. After the final (fortieth) cycle, the sample mixture is incubated at 72°C for ten minutes.

Detection of the PCR amplified taggant is achieved by standard nucleic acid hybridization assays using the capture probe provided in Table 1. The protocol for the hybridization assay is not critical. The means used have been detailed in PCT Publication No. 89/11548, published November 30, 1989. Briefly, the taggant was captured by hybridization to an immobilized probe, GH89 (table 1). The probe was immobilized using ultraviolet irradiation on a positively charged nylon membrane.

A. Gunpowder

Preparation: (a) Add 16ng of taggant to 1 ml of distilled water, (b) mix solution with 1 g of nitrocellulose based gunpowder, and (c) dry in air or under vacuum at 85°C.

Recovery: (a) Wash gunpowder with distilled water (1 ml); (b) add 50 μ l of this solution to PCR reaction mix or place gunpowder flakes directly in 100 μ l PCR reaction mix; and (c) amplify.

B. Oil

Preparation: (a) Mix together 40 μ g of taggant, 10 μ l "Tween® 80" (detergent), 10 μ l "Span® 80" (detergent), and 100 μ l distilled water; and (b) add mixture to 1.7 ml oil. The combination is then thoroughly mixed.

Recovery: Add oil directly to PCR mix, vortex and amplify; or use a standard phenol-chloroform extraction. After treatment with phenol, the taggant was detected in the boundary layer between the oil and water phases.

C. Medicines (water soluble tablets)

Preparation: (a) Add 48 μ g of taggant to 325 μ l of distilled water; (b) add 50 μ l of this mix to a tablet; and (c) allow to dry.

Recovery: (a) dissolve the tablet in 5 ml of H₂O; (b) place 50 μ l of this solution in PCR mix; and (c) amplify.

D. Ink

Preparation: If water insoluble, mix with detergents as for tagging oil. If water soluble, add a dilute concentration of taggant directly to the ink. Recovery: As described for oils and medicines.

E. Paper goods

Preparation: (a) Add dilute solution of taggant 2-3 ng/cm² to paper, and (b) allow to air dry or dry under vacuum.

Recovery: (a) Soak paper in distilled water with or without detergents to aid in solubilization; (b) optionally concentrate taggant; and (c) amplify by standard PCR procedure.

F. Food Stuffs

Preparation: Add 20 ng taggant to each gram of food by physical mixing or spray application.

Recovery: (a) Food material is washed with distilled water; (b) the rinse is optionally concentrated; and (c) the rinse material is subjected to the standard PCR procedure.

Claims

1. A method of monitoring the presence of a substance released into the environment or the stream of commerce, which comprises tagging the substance for its subsequent identification by detection of the taggant, with a nucleic acid of at least 20 and less than 1,000 nucleotides in length which is not a functioning part of the substance, collecting the substance, amplifying said nucleic acid, and detecting said nucleic acid.

2. A method of claim 1 wherein the step of amplifying is achieved using polymerase chain reaction technology.
3. A method of claim 1 or claim 2 wherein the nucleic acid is a synthetic nucleic acid.
- 5 4. A method of claim 3 wherein the synthetic nucleic acid comprises inosine bases or derivatized nucleotides selected from the group consisting of 7-deazo-2'-deoxyguanosine, alkyl- phosphonate oligodeoxynucleotides, phosphorothioate oligodeoxynucleotides, and alpha-anomeric oligodeoxynucleotides.
- 10 5. A method of any of claims 1 to 4 wherein the substance is selected from air pollutants, oils, aromatic compounds, explosive compositions, food stuffs, medicaments, inks, paper goods, and paint products.
6. A method of any of claims 1 to 5 wherein the nucleic acid is covalently linked to a component of the substance or to a solid support mixed with the substance.
- 15 7. A method of any of claims 1 to 5 wherein the nucleic acid is encapsulated by a polymeric substance or a lipophilic composition.
- 20 8. A method of tagging a substance to be released into the environment or the stream of commerce for its subsequent identification by detection of the taggant, comprising the step of incorporating into or onto the substance a nucleic acid having a specific sequence of at least 20 and less than 1,000 nucleotide bases in length which is not a functioning part of the substance, wherein the nucleic acid is a synthetic nucleic acid comprising inosine bases or derivatized nucleotides selected from the group consisting of 7-deazo-2'-deoxyguanosine, alkyl- phosphonate oligodeoxynucleotides, phosphorothioate oligodeoxynucleotides, and alpha-anomeric oligodeoxynucleotides.
- 25 9. A method of claim 8 wherein the substance is selected from air pollutants, oils, aromatic compounds, explosive compositions, food stuffs, medicaments, inks, paper goods, and paint products.
- 30 10. A method of any of claims 8 and 9 wherein the nucleic acid is covalently linked to a component of the substance or to a solid support mixed with the substance.
11. A method of any of claims 8 and 9 wherein the nucleic acid is encapsulated by a polymeric substance or a lipophilic composition.

35 Patentansprüche

1. Verfahren zur Überwachung der Gegenwart einer Substanz, die in die Umgebung oder den Handelsstrom freigesetzt wird, das umfaßt, daß man die Substanz für die nachfolgende Identifizierung durch Nachweis der Markierung mit einer Nucleinsäure mit mindestens 20 und weniger als 1.000 Nucleotiden, die kein funktioneller Teil der Substanz ist, markiert, die Substanz sammelt, die Nucleinsäure amplifiziert und die Nucleinsäure nachweist.
2. Verfahren nach Anspruch 1, worin die Stufe der Amplifikation unter Verwendung der Polymerase-Kettenreaktion erreicht wird.
- 45 3. Verfahren nach Anspruch 1 oder 2, worin die Nucleinsäure eine synthetische Nucleinsäure ist.
4. Verfahren nach Anspruch 3, worin die synthetische Nucleinsäure Inosinbasen oder derivatisierte Nucleotide ausgewählt aus der Gruppe bestehend aus 7-Deazo-2'-deoxyguanosin, Alkylphosphonat-Oligodeoxynucleotiden, Phosphorothioat-Oligodeoxynucleotiden und α -anomeren Oligodeoxynucleotiden umfaßt.
- 50 5. Verfahren nach einem der Ansprüche 1 bis 4, worin die Substanz ausgewählt ist aus luftverschmutzenden Substanzen, Ölen, aromatischen Verbindungen, explosiven Zusammensetzungen, Nahrungsmitteln, Arzneimitteln, Tinten, Papierwaren und Farbenprodukten.
- 55 6. Verfahren nach einem der Ansprüche 1 bis 5, worin die Nucleinsäure kovalent an eine Komponente der Substanz oder an einen festen Träger, der mit der Substanz gemischt ist, gebunden ist.
7. Verfahren nach einem der Ansprüche 1 bis 5, worin die Nucleinsäure in eine polymere Substanz oder eine lipophile

Zusammensetzung eingekapselt ist.

8. Verfahren zum Markieren einer Substanz die in die Umgebung oder den Handelsstrom freigesetzt werden soll für die nachfolgende Identifizierung durch Nachweis der Markierung, das die Stufen umfaßt, daß man in oder auf die Substanz eine Nucleinsäure bringt mit einer spezifischen Sequenz von mindestens 20 und weniger als 1.000 Nucleotidbasen, die kein funktioneller Teil der Substanz ist, wobei die Nucleinsäure eine synthetische Nucleinsäure ist, die Inosinbasen oder derivatisierte Nucleotide ausgewählt aus der Gruppe bestehend aus 7-Deazo-2'-deoxy-guanosin, Alkylphosphonat-Oligodeoxynucleotiden, Phosphorothioat-Oligodeoxynucleotiden and α -anomeren Oligodeoxynucleotiden umfaßt.
9. Verfahren nach Anspruch 8, worin die Substanz ausgewählt ist aus luftverschmutzenden Substanzen, Ölen, aromatischen Verbindungen, explosiven Zusammensetzungen, Nahrungsmitteln, Arzneimitteln, Tinten, Papierwaren und Farbenprodukten.
10. Verfahren nach einen der Ansprüche 8 oder 9, worin die Nucleinsäure kovalent an eine Komponente der Substanz oder einen festen Träger, der mit der Substanz gemischt ist, gebunden ist.
11. Verfahren nach einem der Ansprüche 8 oder 9, worin die Nucleinsäure in eine polymere Substanz oder eine lipophile Zusammensetzung eingekapselt ist.

Revendications

1. Procédé pour contrôler la présence d'une substance libérée dans l'environnement ou le flux du commerce, qui comprend le marquage de la substance pour son identification ultérieure par détection du marqueur, avec un acide nucléique de longueur d'au moins 20 et de moins de 1000 nucléotides qui n'est pas une partie fonctionnelle de la substance, le recueil de la substance, l'amplification et la détection dudit acide nucléique.
2. Procédé selon la revendication 1 dans lequel l'étape d'amplification est effectuée à l'aide de la réaction de polymérase en chaîne.
3. Procédé selon la revendication 1 ou 2 dans lequel l'acide nucléique est un acide nucléique synthétique.
4. Procédé selon la revendication 3 dans lequel l'acide nucléique synthétique comprend des bases inosine ou des nucléotides dérivés choisis dans le groupe constitué de la 7-déazo-2'-désoxyguanosine, des oligodésoxynucléotides alkyl-phosphonate, des oligodésoxynucléotides phosphorothioate, et des oligodésoxynucléotides alpha-anomériques.
5. Procédé selon l'une des revendications 1 à 4 dans lequel la substance est choisie parmi les polluants aériens, les huiles, les composés aromatiques, les compositions explosives, les produits alimentaires, les médicaments, les encres, les produits papier et les produits peinture.
6. Procédé selon l'une des revendications 1 à 5 dans lequel l'acide nucléique est lié de façon covalente à un composant de la substance ou à un support solide mélangé avec la substance.
7. Procédé selon l'une des revendications 1 à 5 dans lequel l'acide nucléique est encapsulé par une substance polymère ou une composition lipophile.
8. Procédé pour marquer une substance destinée à être libérée dans l'environnement ou le flux du commerce pour son identification ultérieure par détection du marqueur, qui comprend l'étape d'incorporation dans ou sur la substance d'un acide nucléique possédant une séquence spécifique de longueur d'au moins 20 et de moins de 1000 bases nucléotidiques qui n'est pas une partie fonctionnelle de la substance, dans lequel l'acide nucléique est un acide nucléique synthétique comprenant des bases inosine ou des nucléotides dérivés choisis dans le groupe constitué de la 7-déazo-2'-désoxyguanosine, des oligodésoxynucléotides alkyl-phosphonate, des oligodésoxynucléotides phosphorothioate, et des oligodésoxynucléotides alpha-anomériques.
9. Procédé selon la revendication 8 dans lequel la substance est choisie parmi les polluants aériens, les huiles, les composés aromatiques, les compositions explosives, les produits alimentaires, les médicaments, les encres, les

EP 0 477 220 B1

produits papier et les produits peinture.

10. Procédé selon l'une des revendications 8 et 9 dans lequel l'acide nucléique est lié de façon covalente à un composant de la substance ou à un support solide mélangé avec la substance.

11. Procédé selon l'une des revendications 8 et 9 dans lequel l'acide nucléique est encapsulé par une substance polymère ou une composition lipophile.

DESIGNATIONS OF "DE"

Until further notice, any designation of "DE" in any international application whose international filing date is prior to October 3, 1990, shall have effect in the territory of the Federal Republic of Germany with the exception of the territory of the former German Democratic Republic.

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CF	Central African Republic	KP	Democratic People's Republic of Korea	SE	Sweden
CG	Congo	KR	Republic of Korea	SN	Senegal
CH	Switzerland	LI	Liechtenstein	SU	Soviet Union
CM	Cameroon	LK	Sri Lanka	TD	Chad
DE	Germany, Federal Republic of	LU	Luxembourg	TC	Togo
DK	Denmark			US	United States of America